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(54) **LYOPHILIZED MONOCLONAL ANTIBODY PREPARATION.**

(57) A lyophilized monoclonal antibody preparation containing gelatin, carboxylic acid or its salt added thereto in order to suppress the denaturation during lyophilization and to attain a persistent antigen binding activity. It can be supplied, like an immunoglobulin preparation, as an immunotherapeutic agent for preventing or treating infectious diseases caused by bacteria, viruses and so forth.

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FIELD OF THE INVENTION

The present invention relates to a freeze-dried preparation comprising a monoclonal antibody(or antibodies) as a main ingredient(s).

PRIOR ART

A monoclonal antibody is a homogeneous globulin protein having reactivity to only a specific epitope. Recent progress in technologies of cell fusion, cultivation and protein purification, etc. has made it possible to produce large amounts of monoclonal antibodies. As a result, monoclonal antibodies have come to be utilized in various fields, such as various analyses, diagnoses, treatments and prophylaxes. In particular, expectations of monoclonal antibodies as medicines for treatments and prophylaxes are increasing. Above all, their application to human bodies is expected to be developed further more in future, and development of human-derived monoclonal antibodies which are favorable in point of antigenicity is being advanced.

Hitherto, in this technical field, polyclonal antibodies such as immunoglobulins have been used for the same purpose for medical diagnosis and treatment. While a monoclonal antibody is a homogeneous one having reactivity to only a specific epitope, a polyclonal antibody is a mixture of plural antibodies as is named so. Therefore, in a polyclonal antibody, plural molecules each having different properties act to mutually stabilize them so that such a polyclonal antibody is, as a whole, in a relatively stable state. However, in a purified monoclonal antibody, stabilization by the interaction between different molecules could not be expected so that such a monoclonal antibody is unstable to various physical and chemical actions irrespective of the globulin class of itself.

Globulin proteins such as monoclonal antibodies and polyclonal antibodies are often heated for the purpose of inactivating viruses therein, especially when they are used for medical diagnosis and treatment. Such globulin proteins are unsuitable to storage in the solutions for a long period of time. Therefore, employment of freeze-drying formulation has been common for a stable storage of such globulin protein molecules. In addition, the globulin proteins are often treated with acids or alkali substances, if desired.

To such heat-treatment, freeze-drying and acid- or alkali-treatment, polyclonal antibodies are generally stable, but monoclonal antibodies would often be denatured and easily lose their activity by such treatment. In particular, IgM is less stable than any other monoclonal antibodies of other globulin classes (e.g., IgG, IgA and IgE). Regarding heat-treatment of antibodies, for example, JP-A 61-76423 (the term "JP-A" means an "unexamined published Japanese patent application") discloses the fact that monoclonal antibodies are unstable to heat-treatment and that, for the purpose of overcoming the thermal instability, a hydrolysate of ovalbumin is added to a monoclonal antibody preparation.

On the other hand, freeze-drying treatment involves a problem specific to monoclonal antibodies. Namely, in freeze-drying a monoclonal antibody, if a monoclonal antibody solution is freeze-dried without adding a stabilizer thereto, there occurs a problem of decrease of the antigen-binding activity of the monoclonal antibody due to denaturation of itself during freeze-drying. Therefore, it is necessary to prevent this problem. The problem is noticeable in freeze-drying a monoclonal antibody, while it is not so significant in freeze-drying a polyclonal antibody as a polyclonal antibody is stable because of the above-mentioned reasons.

In preparing a freeze-dried product of a monoclonal antibody, addition of albumin of a heterologous protein to a solution of a monoclonal antibody before freeze-drying (for example, JP-A 60-146833, 61-78730 and 61-78731, and WO 90/11091) or addition of maltose of a saccharide thereto (for example, WO 89/11297) is known.

Immunoglobulins of polyclonal antibodies are usually used at a relatively low concentration, and aggregates would often form in the solutions during storage or during the succeeding freeze-drying treatment. The aggregates are considered to cause a serious anaphylactoid side effect, when the globulin containing them is intravenously injected to human bodies. Therefore, for the purpose of preventing formation of such aggregates, addition of a heterologous protein to the stock solutions is known. For instance, addition of gelatin alone of a heterologous protein to an immunoglobulin solution (for example, JP-A 58-167518, Vox. Sang. (1983) 51, 81-86) or addition of both sucrose of a saccharide and gelatin thereto is known to be effective for preventing formation of aggregates in the stock solutions and also for maintaining antibacterial and antiviral activities (SU 700132). All the technologies as disclosed above are to prevent formation of aggregates in a high concentration solution of an immunoglobulin of a polyclonal antibody. None of them mention or discuss the matter of decrease of the antigen-binding activity of polyclonal antibodies due to freeze-drying treatment. On the other hand, a monoclonal antibody is stored or freeze-dried in the form having a relatively low concentration. Even under such a low concentration, however, there

is still a problem of denaturation of a monoclonal antibody during freeze-drying as well as a problem of decrease of its antigen-binding activity. The matter has not heretofore been identified as to whether or not addition of gelatin used to prevent formation of aggregates of immunoglobulins would be useful for solving this problem.

On the other hand, it is widely known that carboxylic acids and their salts are used as a component of buffers for pH maintenance of various protein solutions. For instance, WO 89/11298 discloses addition of maltose, sodium chloride or sodium phosphate, as a stabilizer, to a stock solution of a monoclonal antibody for the purpose of preventing formation of aggregates to precipitate in the solution. It also mentions use of sodium citrate, in addition to sodium phosphate, as a component of the buffer. However, it merely indicates a technology of preventing formation of aggregates in a stock solution of a monoclonal antibody during storage, but it does not disclose at all a treatment for freeze-drying a monoclonal antibody, a treatment for preventing denaturation of a monoclonal antibody during freeze-drying it and also a treatment for preventing decrease of the antigen-binding activity thereof. WO 89/11297 discloses a technology of adding maltose, as a stabilizer, to a monoclonal IgG antibody solution to be freeze-dried and further adding, as a buffer component, of 5 to 10 mM sodium acetate thereto so that the pH value of the solution is kept to fall within an acidic range of being from 3 to 6. In this case, sodium acetae is obviously used as a component of a buffer solution. WO 89/11297 suggests nothing as to the fact that carboxylic acid or its salt would still act as a stabilizer for preventing denaturation of antibody during freeze-drying treatment thereof in the pH range where the carboxylic acid or its salt does not have their buffer action. Regarding a pH range of an antibody solution, if an antibody solution having a low pH value is intravenously injected as an injection to a body, it would often have a pain in the injected part thereby. Where an antibody solution is used as an injection, it is desirable to have a pH value in an approximately neutral pH range. However, utilization of such a use of an antibody solution in a neutral pH range is not suggested in WO 89/11297.

For the purpose of inactivating viruses which would contaminate immunoglobulins in preparing them from sera or plasma, immunoglobulins are often heated in the form of their solutions. For instance, JP-A 62-292731, 61-194035, 61-191622 and 57-140724 disclose addition of carboxylic acids to said globulin solutions for this purpose. JP-A 61-78730 and 61-78731 disclose addition of sodium acetate to immunoglobulins and heating them in a dry state. However, all of them merely mention addition of carboxylic acids for the purpose of stabilizing immunoglobulins in the heat-treatment. It has not heretofor been known whether or not carboxylic acids and their salts would be useful for preventing denaturation of antibodies during freeze-drying of them and also for preventing decrease of an antigen-binding activity owing to said denaturation.

PROBLEM TO BE SOLVED BY THE INVENTION

The object of the present invention is to provide stable freeze-dried preparations of monoclonal antibodies, which are free from denaturation of the monoclonal antibodies during freeze-drying of them and from decrease of their antigen-binding activity owing to said denaturation.

MEANS FOR SOLVING THE PROBLEM

The present inventors earnestly studied for the purpose of solving the above-mentioned problem and, as a result, have found that gelatin, carboxylic acids or their salts are effective for stabilizing a monoclonal antibody in freeze-drying it Namely, they have found that, by addition of gelatin to a solution containing a monoclonal antibody to be freeze-dried, denaturation of the monoclonal antibody during freeze-drying it as well as decrease of its antigen-binding activity may be prevented and that, by addition of carboxylic acid or its salt to a solution containing a monoclonal antibody to be freeze-dried, denaturation of the monoclonal antibody during freeze-drying it as well as decrease of its antigen-binding activity owing to said denaturation may be prevented in a broad pH range and even at a pH value being outside the range where a buffer action is exhibited. On the basis of these observations, they have found that it is possible to prepare a stable and highly safe preparation composition of a monoclonal antibody, and have completed the present invention.

Specifically, the present invention provides a freeze-dried preparation containing a monoclonal antibody and geltain as well as a preparation as prepared by freeze-drying a solution containing a monoclonal antibody and carboxylic acid or its salt and having pH between 6.1 and 8.1.

Next, the present invention will be explained concretely hereunder.

The monoclonal antibody to be used in the present invention is any and every monoclonal antibody that is generally obtained from human beings, mice, rats and others, and the origins and the producing means

are not specifically defined. For instance, the monoclonal antibody for use in the present invention may be obtained from a culture medium as obtained by cultivating antibody-producing cells as prepared by known methods such as cell fusion method or transformation method, or by cultivating cells into which a cloned antibody gene has been incorporated, or from ascites, etc. of a mouse into which such antibody-producing cells have been transplanted. For purifying the monoclonal antibody obtained from such a cell culture medium or mouse ascites or the like, usable are various purification methods such as ammonium sulfate salting-out, ion exchange chromatography, gel filtration, affinity chromatography, ultra-centrifugation, adsorption chromatography and hydrophobic chromatography. The globulin classes of the monoclonal antibody to be used in the present invention are mostly IgG, IgM, IgA and IgE, but they are not specifically defined. A monoclonal antibody of any globulin class can be used in the present invention. Above all, IgM is less stable than those of other globulin classes. Therefore, the stabilizing method effective to IgM class monoclonal antibody may easily be applied to monoclonal antibodies of other globulin classes. In the present invention, a single monoclonal antibody may be used or plural monoclonal antibodies may be also used as a mixture of them with no problem.

Gelatin may be grouped into two types (neutral type and acidic type) according to the methods of preparing it, of which the isoelectric points are different from each other. Both of them may be used in the present invention. In addition, chemically modified gelatins such as oxypolygelatin or modified liquid gelatins may be also used.

As carboxlyic acid, usable are, for example, citric acid, acetic acid, oxalic acid, succinic acid and fumaric acid. Citric acid is preferable. As salt of carboxylic acid, usable are, for example, sodium citrate, potassium citrate, sodium acetate, potassium acetate, sodium oxalate, potassium oxalate, sodium succinate, potassium succinate, sodium fumarate and potassium fumarate. Sodium citrate is preferable.

For the purpose of stabilizing the monoclonal antibody or for the purpose of pH adjusting, isotonicating and buffering the monoclonal antibody-containing solution to be freeze-dried, inorganic salt, monosaccharide, disaccharide, sugar alcohol or amino acid may be further added, in addition to gelatin or carboxylic acid or its salt.

As inorganic salt, usable are, for example, sodium chloride, potassium chloride and magnesium chloride. Sodium chloride is preferable.

As monosaccharide, usable are, for example, glucose, mannose, galactose and fructose. Glucose or mannose is preferable.

As disaccharide, usable are, for example, maltose, sucrose and lactose. Maltose or sucrose is preferable.

As sugar alcohol, usable are, for example, sorbitol and mannitol. Mannitol is preferable.

As amino acid, usable are, for example, glycine, alanine, valine, leucine, isoleucine, tyrosine, phenylalanine, serine, threonine, glutamine, glutamic acid, asparagine, aspartic acid, arginine, lysine, histidine, proline, tryptophan, methionine and cysteine. Glycine or arginine is preferable.

For producing the freeze-dried preparation of the present invention, a monoclonal antibody solution containing gelatin or carboxylic acid or its salt may be freeze-dried. Preferably, a monoclonal antibody solution is added to a buffer containing gelatin or carboxylic acid or its salt and having an adjusted pH value; or gelatin or carboxylic acid or its salt is added to a monoclonal antibody-containing solution. The concentration of the monoclonal antibody in the solution to be used in the present invention is from 0.01 mg/ml to 50 mg/ml, preferably from 0.1 mg/ml to 10 mg/ml. The addition amount of gelatin is from 1/100 to 100 parts by weight to one part by weight of the monoclonal antibody. Preferably, it is from 1/10 to 10 parts by weight to one part by weight of the same. The concentration of carboxylic acid or its salt to be added is from 2 mM to 500 mM, preferably from 10 mM to 200 mM.

The pH value of the solution of dissolving the monoclonal antibody to be freeze-dried is from 4.0 to 8.1 when gelatin is added thereto; or it is from 6.1 to 8.1, preferably from 6.5 to 7.8, when carboxylic acid is added or both gelatin and carboxylic acid are added. Adjustment of the pH value of the solution may be done by use of organic acids, inorganic acids, inorganic salts or other compounds which are generally used for pH adjustment, singly or in combination of two or more of them. As compound usable for such pH adjustment, there are mentioned, for example, citric acid, sodium citrate, potassium citrate, phosphoric acid, sodium phosphate, potassium phosphate, hydrochloric acid, tris(hydroxymethyl)aminomethane, acetic acid, sodium acetate, potassium acetate, sodium hydroxide, boric acid, sodium borate, and potassium borate. The concentration of the buffer of dissolving the monoclonal antibody is from 5 mM to 500 mM, preferably from 10 mM to 500 mM. As mentioned above, carboxylic acid or its salt may be also used for pH adjustment of a monoclonal antiboy containing solution, and the above-mentioned amount of the acid or its salt indicates all the amount thereof in the solution including one for pH adjustment.

The thus prepared monoclonal antibody solution may be well stable when freeze-dried directly as it is. It is also possible to add thereto a surfactant such as Tween 20 or Tween 80, a human or bovine albumin, or a chelating agent such as EDTA, for the purpose of isotonicating the solution or of preventing adhesion of the monoclonal antibody to the container containing the solution.

Freeze-drying of the monoclonal antibody solution may be carried out by any ordinary known method, and the drying temperature and the vacuum degree in the method may be selected suitably.

EXAMPLES

Next, the present invention will be explained by way of the following examples, which are, however, not limitative. IgM is exemplified herein as a monoclonal antibody in the present invention. This is because, as mentioned above, IgM is less stable than antibodies of other globulin classes (e.g., IgG, IgA and IgE), and therefore the stabilizing effect to be verified in IgM may be easily applied to antibodies of other globulin classes.

Example 1:

Cells of Epstein-Barr virus (EB virus) transformed cell line MP-5038 (FERM BP-1596) reactive to Group E serotype Pseudomonas aeruginosa were cultured, and a human monoclonal antibody was purified from the culture supernatant by ammonium sulfate salting-out, gel filtration with Sephacryl S-300 (Pharmacia Co.) and column chromatography with a hydroxyapatite HPLC column (Mitsui Toatsu Chemicals, Inc.) and Blue-Sepharose (Pharmacia Co.). The monoclonal antibody as obtained by these methods had a purity of 99% or higher, as analyzed by SDS-electrophoresis and HPLC with a gel filtration column. The monoclonal antibody was dissolved in a phosphate-buffered physiological saline having an adjusted pH value of 7.4 (hereinafter referred to as PBS), to have a final concentration thereof of being 0.1 mg/ml. On the other hand, gelatin (high-grade gelatin; Nippi Co., type A (neutral gelatin) and type B (acidic gelatin)) was added thereto to have a final concentration thereof of being from 0.001 to 1%. The resulting solution was then put in 2 ml-volume polypropylene cryotubes (Corning Co.) under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. After dried, the same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured by the method mentioned below.

(Method of Measuring Antigen-Binding Activity)

Measurement of the antigen-binding activity of the anti-Pseudomonas aeruginosa antibody was carried in the manner mentioned below. A lipopolysaccharide (LPS), as prepared from formalin-killed cells of Group E serotype Pseudomonas aeruginosa ATCC 27581 by Tanabe et al's method (Menekijikkensousahou C, (1978) 1793-1801), was dissolved in PBS to have a concentration of 1 mg/ml, and this was diluted by 500-fold with 0.1 M phosphate buffer (pH 7.0). The thus diluted solution was then put in wells of a 96-well EIA plate (Immulon-600; Greiner Co.) in an amount of 50 µl/well. The plate was allowed to stand at 4°C overnight for coating, and it was then washed with PBS containing 0.05% Tween 20 (hereinafter referred to as a "washing solution"). A PBS containing 0.5% bovine serum albumin (hereinafter referred to as a "blocking solution") was added to each well in an amount of 200 µl/well, and the plate was then shaken at room temperature for one hour so that the non-specific protein-binding sites were saturated. After the blocking solution was removed, solutions of a sample to be tested, each having a multi-fold diluted concentration in order from a determined concentration, were put in the wells each in an amount of 100 µl/well, and the plate was then shaken for 2 hours at room temperature. After washed with the washing solution four times, a peroxidase-labeled goat anti-human IgM antibody (Tago Co.) was diluted by 1000-fold with the blocking solution, and was put in each well in an amount of 100 µl/well and the plate was shaken at room temperature for 2 hours. After washed with the washing solution four times and then with 0.1 M citric acid buffer (pH 4.0) one time, a substrate solution containing 1 mg/ml of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and 0.003% hydrogen peroxide in the same buffer was put into each well in an amount of 50 µl/well, and the plate was then shaken at room temperature. After 30 minutes, 2% succinic acid was added to each well in an amount of 50 µl/well so that the enzymatic reaction therein was stopped. The absorbance at 414 nm was measured with a 96-well plate reader (Nippon InterMed Co.). Double logarithmic plotting was done between the reciprocal of the diluting magnification and the absorbance, and the diluting magnification to show the absorbance of being 0.1 was obtained and indicated as

the antigen-binding activity of the sample tested.

The results are shown in Table 1, as a relative activity on the basis of the antigen-binding activity of the sample before frozen of being 10. Where a monoclonal antibody was freeze-dried without addition of gelatin thereto, the antigen-binding activity noticeably decreased. As opposed to the case, when gelatin was added, the antigen-bindability was well recovered even in the freeze-dried product, and the effect depended upon the concentration of the gelatin added.

Table 1

Gelatin Concentration (%)	Antigen-Binding Activity	
	Neutral Gelatin	Acidic Gelatin
0	2	2
0.001	4	3
0.003	6	6
0.01	10	8
0.03	10	10
0.1	10	10
1	10	10

Example 2:

The same monoclonal antibody as that used in Example 1 was dissolved in various buffers each having a different pH value to each have a final concentration of 0.1 mg/ml. On the other hand, a neutral gelatin was added to them to each have a final concentration of 0.01%. Each of them was put in polypropylene cryotubes each in an amount of 0.5 ml under a sterilized condition and frozen at -80°C. These were then freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved, and the antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

The results obtained are shown in Table 2, as a relative activity based on the activity of the sample before frozen of being 10. At every pH condition, the antigen-binding activity was well recovered.

Table 2

Buffer (0.2 M)	pH	Gelatin Concentration (%)	Antigen-Binding Activity
Sodium Citrate	4.0	0.01	10
	5.0	0.01	10
	6.0	0.01	10
Sodium Phosphate	6.2	0.01	10
	7.0	0.01	10
	8.1	0.01	10

Example 3:

0.1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in 20 mM phosphate buffer (pH 7) not containing or containing 2 or 10 mM sodium citrate, whereupon the salt concentration of the resulting solution was adjucted to be 150 mM with sodium chloride. The monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

The results obtained are shown in Table 3, as a relative activity based on the activity of the sample before frozen of being 10. Where the monoclonal antibody was freeze-dried in the absence of sodium citrate, the antigen-dinding activity of the freeze-dried monoclonal antibody noticeably decreased. As opposed to the case, when the monoclonal antibody was freeze-dried in the presence of sodium citrate, then the antigen-dinding activity of the freeze-dried monoclonal antibody was well recovered, depending upon the concentration of the sodium citrate as added.

Table 3

Sodium Citrate Concentration (mM)	0	2	10
Antigen-Binding Activity	2	5	9

Example 4:

0.1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in 50 mM phosphate buffer (pH 6.1 to 8.1) containing sodium citrate in a concentration of from 10 mM to 200 mM, whereupon sodium chloride was added thereto, if necessary, so that the salt concentration of the resulting solution became 150 mM. The resulting monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vaccuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

The results obtained are shown in Table 4, as a relative activity based on the activity of the sample before frozen of being 10. By adding sodium citrate at pH of from 6.1 to 8.1, the antigen-binding activity of all the freeze-dried products was well recovered.

Table 4

pH of Solution	Antigen Binding Activity Sodium Citrate Concentration (mM)			
	10	50	100	200
6.1	10	10	10	10
7.0	9	10	10	10
8.1	9	10	10	-

Example 5:

0.1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in PBS. In addition, 0.003%, as a final concentration, of a neutral gelatin was added thereto, and further glucose, sucrose, mannitol, glycine or arginine was added thereto in an amount, as a final concentration, of from 0.001 to 0.1%. The resulting monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

The results obtained are shown in Table 5, as a relative activity based on the activity of the sample before frozen of being 10. The antibody activity was well recovered in all cases of the low molecular substances, depending upon the concentration of them.

Table 5

Concentration of Gelatin(%)	Concentration of Low Molecular Substance (%)	Antigen-Binding Activity				
		glucose	sucrose	mannitol	glycine	arginine
0.003	0.001	7	7	7	8	7
0.003	0.003	8	8	7	8	8
0.003	0.01	8	6	8	10	8
0.003	0.03	8	8	8	10	8
0.003	0.1	8	10	8	10	8
0.003	0	8	6	6	6	6

Example 6:

0.1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in PBS. In addition, 0.003%, as a final concentration, of a neutral gelatin was added thereto, and further 0.5 or 1%, as a final concentration, of mannitol was added thereto. The resulting monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

The results obtained are shown in Table 6, as a relative activity based on the activity of the sample before frozen of being 10. The antigen-binding activity was well recovered in all the freeze-dried products, each containing a different concentration of mannitol.

Table 6

Concentration of Mannitol (%)	0.5	1.0
Antigen-Binding Activity	10	10

Example 7:

1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in 0.1 M phosphate buffer (pH 7.0) containing a neutral gelatin (0.01%), sodium citrate (0.02 M), mannitol (0.5%) and sodium chloride (0.05 M). The resulting monoclonal antibody solution was then put in 10 ml-volume glass vials (Iwaki Glass Co.) under a sterilized condition, each in an amount of 1 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before frozen.

Example 8:

1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%). The resulting monoclonal antibody solution was then put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured in the same manner as in Example 1. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before frozen.

Example 9:

Cells of human-human hybridoma MP5121 (FERM BP-2270) producing a human IgM reactive to Group A serotype Pseudomonas aeruginosa, which had been produced by cell fusion, were cultured, and the monoclonal antibody was purified from the culture supernatant in the same manner as in Example 1. The monoclonal antibody was dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%), to have a final concentration thereof of being 1 mg/ml. The resulting monoclonal antibody solution was then put in glass vials and frozen therein at -80 °C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured in the same manner as in Example 1, provided that the antigen LPS was extracted from Group A serotype Pseudomonas aerugionsa (ATCC 27577). As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before frozen.

Example 10:

Monoclonal antibodies were purified from culture supernatants of cells of human IgM-producing human-human hybridoma MP5097, MP5139, MP5114 and MP5156 (FERM BP-2268, 2272, 2269 2339, respectively), all of which had been produced by cell fusion. These monoclonal antibodies had reactivity with Pseudomonas aeruginosa and were reactive to Groups B, E, G and I serotypes Pseudomonas aeruginosa, respectively. Five kinds of monoclonal antibodies comprising 4 kinds of these monoclonal antibodies and the monoclonal antibody used in Example 9 were dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%), each in an amount, as a final concentration, of 5 mg/ml. These monoclonal antibody solutions were put in glass vials and frozen therein at -80 °C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to each of the freeze-dried products so that each product was dissolved. The antigen-binding activity of each of the monoclonal antibodies in the resulting solutions was measured in the same manner as in Example 1, provided that as the antigen to each antibody, used were LPSs as extracted from ATCC 27577 (to Group A serotype), ATCC 27578 (to Group B serotype), ATCC 27581 (to Group E serotype), ATCC 27584 (to Group G serotype) and ATCC 27586 (to Group I serotype), respectively. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity to the five Pseudomonas aeruginosa LPSs of different serotypes, respectively, as that of the samples before frozen.

ADVANTAGE OF THE INVENTION

In accordance with the present invention characterized by addition of gelatin or carboxylic acid or its salt to a monoclonal antibody-containing solution to be freeze-dried, denaturation of a monoclonal antibody during freeze-drying can be well prevented so that a monoclonal antibody-containing freeze-dried preparation having a stable antigen-binding activity can be provided. The present invention may be applied to a monoclonal antibody of any globulin class, including IgG, IgM, IgA and IgE. Especially, it can be sufficiently applied to unstable IgM. The present invention may be well applied to any of human-derived, mouse-derived and rat-derived monoclonal antibodies. The number of the kinds of the monoclonal antibodies to be contained in the freeze-dried preparation of the present invention may be one or more.

The monoclonal antibody-containing freeze-dried preparation of the present invention may be used, like other immunoglobulin preparations, as an adminiculum for immunoauxotherapy for prophylaxis and treatment of bacterial infectious diseases and viral infectious diseases.

Claims

1. A freeze-dried preparation comprising a monoclonal antibody and gelatin.

2. A preparation which is prepared by freeze-drying a solution comprising a monoclonal antibody and carboxylic acid or its salt and having pH of from 6.1 to 8.1.

3. The preparation as claimed in claim 2, in which the carboxylic acid is citric acid.

4. The preparation as claimed in claim 1, which is obtained by freeze-drying a solution having pH of from 4.0 to 8.1.

5. The preparation as claimed in anyone of claims 1 to 4, in which the monoclonal antibody is a human-derived one.

6. The preparation as claimed in anyone of claims 1 to 4, in which the monoclonal antibody is one having a globulin class of IgM.

7. The preparation as claimed in claim 1 or 4, in which the gelatin is a neutral gelatin or an acidic gelatin.

8. The preparation as claimed in claim 1 or 4, which further contains carboxylic acid or its salt or an inorganic salt.

9. The preparation as claimed in anyone of claims 1 to 4, which further contains at least one of monosaccharide, disaccharide, sugar alcohol and amino acid.

10. The preparation as claimed in anyone of claims 1 to 4, which contains plural monoclonal antibodies.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP92/00226

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] A61K39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61K39/395

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [9]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	JP, A, 58-167518 (Nippon Sekijuji-sha), October 3, 1983 (03. 10. 83), (Family: none)	1, 4-10
Y	JP, A, 60-146833 (The Green Cross Corp.), August 2, 1985 (02. 08. 85), (Family: none)	1, 4-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
April 28, 1992 (28. 04. 92)	May 19, 1992 (19. 05. 92)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	